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Evolution of Bacterial and Fungal Flora during In-Vessel Composting of Organic Household Waste under Air Pressure

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Abstract

This study aims to evaluate the performance of a laboratory-scale in-vessel composting of the organic fraction of municipal solid waste in Morocco. The bioreactor was specifically designed and used for this study and was operated in semi-continuous under air pressure. The studied parameters included operational indices, compost maturity indices, changes in bacterial and fungal flora, and final compost quality. Results showed that the organic fraction of municipal solid waste could be composted successfully in 60 days, revealing a vigorous microbial activity. The final compost was satisfactory for its agricultural application.

1. Introduction

In Morocco, the total generation of solid waste is 6.852 million metric tons [1]. The generation of urban waste is currently about 0.67 kilos per day per capita, considering that the generation of rural waste is about 0.3 kilos per day. Urban solid waste collection is regular and almost daily, estimated at 5.5 million T per year. In 2013, urban solid waste collection covers about 74% of the waste generated in urban areas. However, only less than 1% of the total waste is composted and yet the household waste is characterized by the predominance of fermentable waste Figure 1(vegetable and kitchen waste) and by their high humidity [2]. As a result, composting of local household waste is the most promising technique compared to other disposal routes such as incineration.

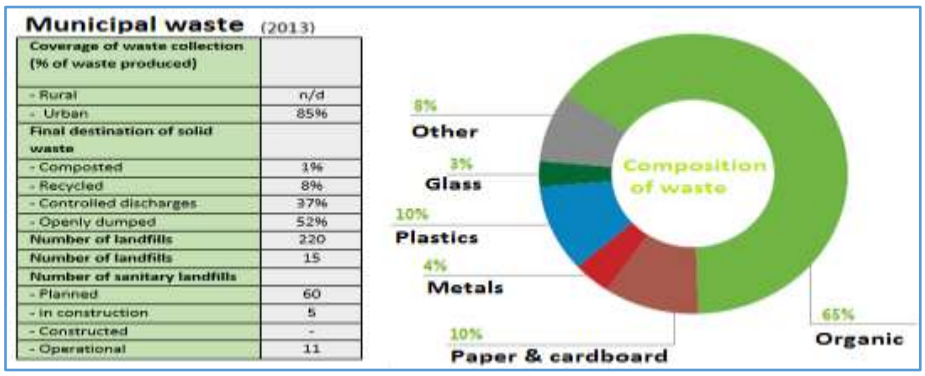

Figure 1: Municipal waste in Morocco.

One of the basic elements of composting household waste is the removal of the unwanted fraction (plastics, scrap metal, etc.) and maintaining the high temperatures required for (i) destruction of pathogens, (ii) stabilization and Humification of fresh organic matter, and (iii) reduction of refuse mass and volume [3-6].Composting is an aerobic process of degradation of organic compounds by the successive action of microorganisms (bacteria, yeasts, and fungi), whose concentration can reach millions, or billions per gram of

compost [7-9]; Their evolution is according to the stages of composting with a profile defined according to the species. Their evolution is mainly related to variations in physicochemical parameters and to the nature and structure of the substrates [10].
Three categories of basic technologies were used for composting: swath, static stack and tank. Each technology can be used successfully to convert biologically into compost under aerobic conditions. The difference between these technologies is the level of process control and the requirements of the terrestrial space. Thus, research should focus on simple and efficient technologies, but requires less space and less energy. The tank composting system has advantages over static and swath systems because it requires less space and better control [11]. Alarge number of documents in the literature on these technologies have been reported [12].
In this study, we intend to study the effect of physico-chemical parameters on the evolution and succession of the endogenous microflora during tank composting.

2. Materiel and methods

2.1. *Description of the bioreactor (design)*
Figure 2 shows the laboratory bioreactor that was specially designed and used for composting waste in this study. The bioreactor is a horizontal metal with a volume of about 200 liters. The thickness of the metal is 1 mm. The bioreactor is designed with an opening in the upper face for the introduction of waste. The bioreactor is equipped with a valve to inject and one to remove the air and a cock for the lixiviat. The sealingisensured by a rubberseal.

Figure 2: Bioreactor at laboratory scale.

2.2. Composition of waste to be composted and experimental design
Based on Moroccan consumption, organic waste or green waste to be compost will consist of 37% vegetable waste, 35% fruit waste, 13% tea waste products and 15% other waste.
A 20kg sample was weighed and manually chopped into small pieces 2 to 5 mm long. The waste obtained, homogenized with 1 kg of potting soil for the supply of the microflora, was transferred to the bioreactor. Moisture adjustment was achieved either by adding the required amount of water or by drying the mixture, under the sun's rays, if there is excess moisture.
The bioreactor was fed with air every day and the gases formed during the degradation process were evacuated at the end of the day. This process is repeated until the compost is stabilized. The composting process took about 30 days until stabilization. Finally, the values of the necessary indicators of the process were measured.

2.3. Methods of analysis of physico-chemical parameters
2.3.1. Temperature
Temperature was measured using a Multi-System digital thermometer (ST-9283B, Indiamart, Delhi, India).

2.3.2. Humidity
The moisture content was measured after the sample was dried at 105 ° C overnight.

2.3.3. Organic material
The organic matter (OM) was calculated from the ash after drying a 20g dry weight sample at 550 ° C for 6 h.

2.3.4. Total organic carbon and total nitrogen kjeldahl
Total organic carbon (TOC) and total nitrogen Kjeldahl (NTK) were measured by the Walkley-Black method and the Kjeldahl method, respectively. The C / N ratio is then calculated as a function of the TOC and NKT concentration.

2.3.5. pH and conductivity

PH and electrical conductivity (EC) were measured using a sample-water mixture (weight: volume = 1: 10). The values were read respectively on the pH-522 WTW meter (Xylem brand, Weilheim, Germany) and on EC-214 conductivity meter (HANNA Instruments, Agadir, Morocco).

2.3.6. Ammonium and nitrate

Ammonium NH_4^+ nitrogen was measured by spectrophotometry of salicylic acid and sodium hypochlorite [13]. Nitrate NO3- was determined using ion chromatography [14].

All analyzes were tripled to ensure reproducibility and representativeness of the sample.

2.4. Methods of analysis of microbiological parameters

10 g of dry compost (after drying at 30 ° C. overnight) are added aseptically to a 250 ml Erlenmeyer flask containing 90 ml of sterile distilled water. This mixture is mechanically stirred with magnetic bars for 30 minutes in order to release the maximum of the microbial charge. The suspension obtained corresponds to dilution 10^{-1}. 10 ml of dilution 10^{-1} are removed aseptically and placed in 90 ml of sterile distilled water thus giving the dilution 10-2 which is stirred for two minutes before taking 10 ml which is added to 90 ml of water Distilled sterile and so on until dilution 10-8. 1 ml is taken from each dilution, working from dilution 10^{-8} at dilution 10^{-1}, and seeded onto the various culture media, using a sterile glass stand.

2.4.1. Total bacterial microflora

The total aerobic mesophilic (TAMF) and thermophilic (TF) flora were counted by counting the colonies on the Plate Count Agar medium and incubated at 35 ° C and 55 ° C respectively.

2.4.2. Fungalmicroflora

The analysis was carried out according to the suspension-dilution technique as described by Rapilly [15]. The culture is carried out on the specific solid medium PDA supplemented with an antibiotic chloramphenicol (Sigma) at the rate of 5 µg / ml. The plates are incubated at 30 ° C. in the dark for 3 days after which the colonies are counted and then the plates are placed under continuous white light in order to promote colony pigmentation while noting the appearance of new colonies. Expression of the results: The determination of the fungal charge is made by counting the colonies and the results are expressed in CFUs (number of Forming ColoniesUnits)/g of compost according to the mathematical formula below. Only boxes containing 15 to 30 colonies at two successive dilutions are retained for enumeration [16].

$$N = \frac{\Sigma \text{ colonies}}{V \times (n1 + 0{,}1\ n2) \times d1}$$

Where:

N: Number of CFU per gram of compost;
Σcolonies: sum of colonies of interpretable boxes;
V: Volume of solution deposited (1 ml);
n1: Number of boxes considered at the first dilution retained;
n2: Number of cans considered at the second dilution retained
and d1: Factor of the first dilution retained.

2.5. Phytotoxicity tests

In this study, two phytotoxicity tests were used. The germination test and the germination index test on different crops (wheat, tomato, lettuce) with the compost produced.

2.5.1. Germination test

This test is based on the germinative power of the seeds of two plants: wheat and tomato. It consists in sowing the same number (10) of seeds in pots containing different percentages of composts and sands. The germination rate is evaluated relative to the control (100% sand). The different proportions of the composts and sands are: 100% S, 75% S + 25% C, 50% S + 50% C, 25% S + 75% C, 100% C (S: sand and C: compost). The different germination conditions for wheat and tomato seeds are listed in Table 1.

2.5.2. Germination index (GI) [17]

The principle consists of placing lettuce seeds in a series of Petri dishes with filter paper soaked in increasing doses of compost extract in parallel to a control series (without compost extract). The whole is placed in a germination chamber (incubator) at 27 ° C. for 24 hours.

Table 1: Seed germination conditions (wheat and tomato).

Seeds	T (°C) possible germination	T (°C) Ideal germination	Soak in water(Hour)	Germination(day)
Wheat	20	20	12	2-3
Tomato	21	21	12-20	7-8

At the end of germination, the seeds are counted and the root lengths are measured. The germination index (GI) is calculated by the following equation:

$$IG = (GB / GT) \times (LB / LT) \times 100$$

With, GI: Germination Index,
GB: Number of germinated seeds in the case of compost,
GT: Number of germinated seeds in the control treatment,
LB: Root length in the case of compost,
LT: Root length for control treatment.

The different doses of compost extracts and distilled water used in this experiment were as follows: 100% E; 75% E + 25% CE; 50% E + 50% CE; 25% E + 75% CE; 100% CE (E: Water, CE: compost extract).

3. Results and discussion

3.1. Evolution of Temperature

Temperature monitoring provides an indirect measure of the intensity of aerobic degradation. At temperatures below 20 ° C, only the psychrotrophic micro-organisms are active. Between 20 and 40 ° C, it is the turn of the mesophilic ones, but the thermophilic microorganisms are active only at temperatures between 40 and 70 ° C [18].

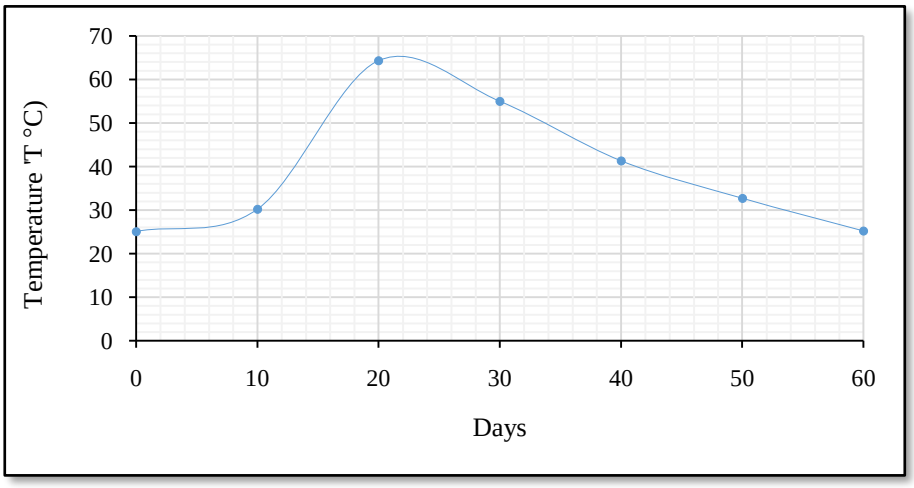

Figure 3: Evolution of temperature during the composting process

At the beginning of composting, biological activity takes place through mesophilic microorganisms, which increases the temperature from 25.1 ° C to 30.2 during the 10th day (Figure3). This increase in temperature is the direct consequence of the oxidation of the organic matter of the substrates [19, 20]. This oxidation thus allows the release of the energy contained in the chemical bonds of the constituent molecules [21].

With composting, the intense activity of the microorganisms generates a temperature increase to 64.3 ° C which is reached on the 20th day and lasts about 6 days which allows the installation of thermophilic and thermo-tolerant microorganisms [22, 23].

However, during the maturation phase that follows the thermophilic phase, heat losses by exchange with the external environment and decrease in nutrients result in a gradual temperature drop and approach the ambient temperature of 25.2 ° C 60 days of composting.

3.2. Evolution of pH

pH is an important factor influencing most biochemical reactions catalyzed by enzymes which allows the bioavailability of nutrients and the solubility of mineral elements for microorganisms.The composting process is characterized by an initial pH of 7.5. The evolution of the pH as a function of time shows three different phases

(Figure 4). Composting passes through a 20-day acidogenic phase, where the pH is around 5.4. Towards the 40th day, there is a rapid passage through a phase of neutrality. On the 50th day, there is an alkalization phase where the pH reaches 7.8. This phase is the result, on the one hand, of an ammonia production from the degradation of protein amines during the ammonification process [24, 25], and on the other hand of a release of the bases previously integrated into the material organic. This stability is also the consequence of the presence of Ca2 + ions which increase during composting after humification and which act as a buffer in the medium [26]. The slightly alkaline final pH 8.1 makes compost a safe product for soil and plants. The pH value obtained in this experiment is consistent with the work of He et al. [27] and Hellmann et al. [28].

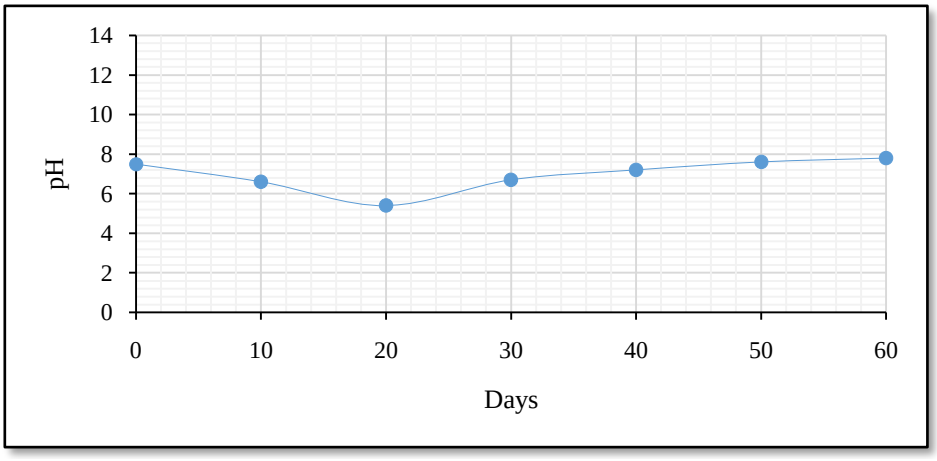

Figure 4: Evolution of pH during the composting process

3.3. Evolution of moisture

Good moisture is essential to increase the activity of micro-organisms, which accelerates the composting process. The moisture content decreases significantly with time, about 70% in the young compost, and only 10% in the mature compost (Figure 5). This loss of water is attributed to leaching and evaporation due to the rise in temperature due to intense microbial activity during composting [2].

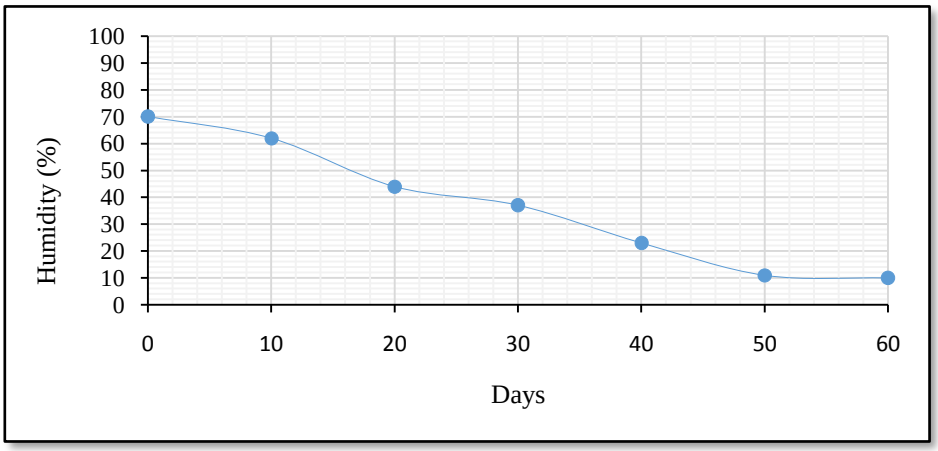

Figure 5: Curve of variation of moistureduring the composting process

3.4. Evolution of NH_4^+ and NO_3^-

NH_4^+ content in the feedstock first increases to the value of 1363 mg / kg during the first 20 days of composting and then decreases progressively (Figure 6), which could be explained by the microbial decomposition of Nitrogen to convert to ammonia. The decrease in NH_4^+ is an indicator of a good process of maturation. Zucconi and Bertoldi [19] recommended a maximum level of $NH_4^+$of 400 mg / kg in mature compost. Thus, the NH_4^+value in the final compost obtained thus corresponds to the demand for agricultural applications.The NO_3^- content maintained a growth trend during the composting process. As the high temperature (over 40 ° C) inhibited the activity and growth of nitrifying bacteria in the thermophilic phase, there was no significant increase in NO_3^-content during the initial phase of the composting process. When the curing process began after the 20th day, a rapid increase in NO_3^- content was observed. After this, the curing process was completed on the 50th day and the NO_3^-content tended to stabilize relatively to reach 1093 mg / kg at the end of composting.

3.5. Evolution of C/N and organic matter OM

According to Figure 7, the C/N ratio shows a gradual decline due to mineralization of organic matter. The initial substrate has a C/N of 27. As soon as the readily available carbon compounds have been exhausted, the C/N reduction rate decreases. This decrease can be explained by the fact that microorganisms consume more carbon (the main component of organic molecules) than nitrogen. At the beginning of composting the C/N ratio was about 27 and the end of the process was reduced to 11.

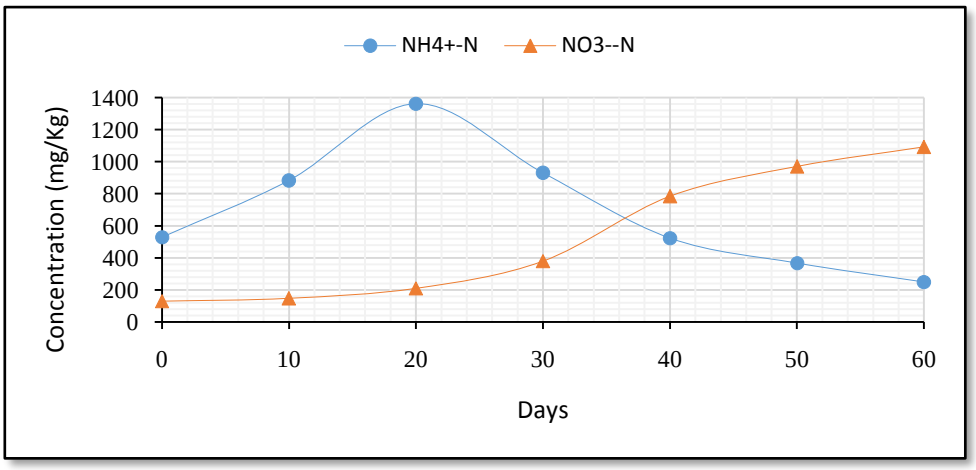

Figure 6: Evolution of NH_4^+ and NO_3^- during the composting process

Hirai et al., [29] proposed a C/N ratio equal to or less than 20 as a standard for mature compost. So, the resulting compost can be qualified as a good quality compost that can be applied in agricultural land. The organic fraction was mineralized into stable compounds by microbial activity, which explains its decrease during the composting process, from about 92% to about 48% after 60 days of composting.

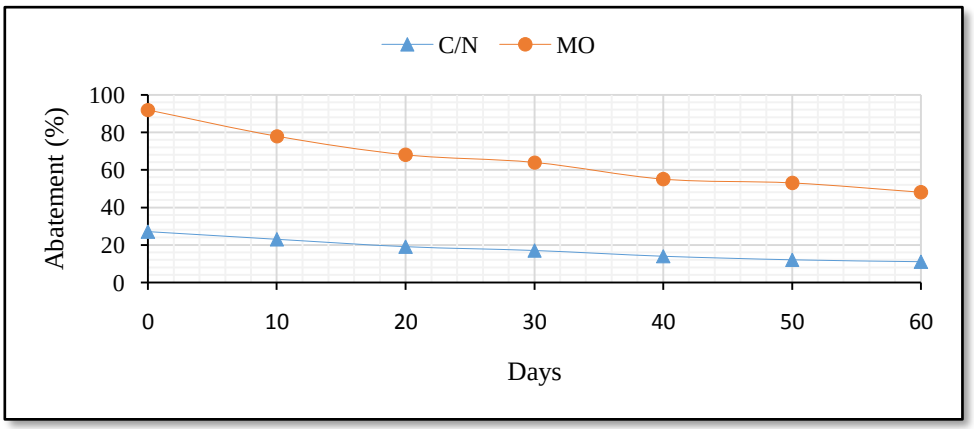

Figure 7: Evolution of C/N and organic matter OM during the composting process

3.6. Evolution of theelectrical conductivity EC

The electrical conductivity (EC) reflects the degree of salinity of the compost produced and indicates its possible phytotoxic / inhibitory effects on plant growth used as fertilizer (eg low germination rate, wilting, etc.) [30]. Compost with a low CE can be used directly while the high CE compost must be thoroughly mixed with soil or other low CE materials before it can be used for crops [31]. According to Figure 8, the EC content curve represents an increase from the initial value of 4.9 ms/cm to a maximum of 7.5 ms/cm on the 20th day, followed by a gradual decrease to at the end of the composting process. The initial EC increase could be caused by the release of inorganic salts such as phosphates and ammonium ions by the decomposition of organic substances [3]. During the composting process, the volatilization of ammonia and the precipitation of mineral salts may be the possible reasons for the decrease of the EC until the final composting phase [32]. The EC of the final compost product does not exceed the limit content of 3 ms/cm, indicating that EC would not adversely affect plant growth [33].

3.7. Evolution of microbialflora

Composting is an aerobic process of degradation of organic compounds by the successive action of microorganisms (bacteria and fungi) whose concentration can reach millions per gram of compost [7-8]; Their

evolution depends on the stages of composting with a defined profile depending on the species, the nature and structure of the substrates and mainly related to variations in physico-chemical parameters.

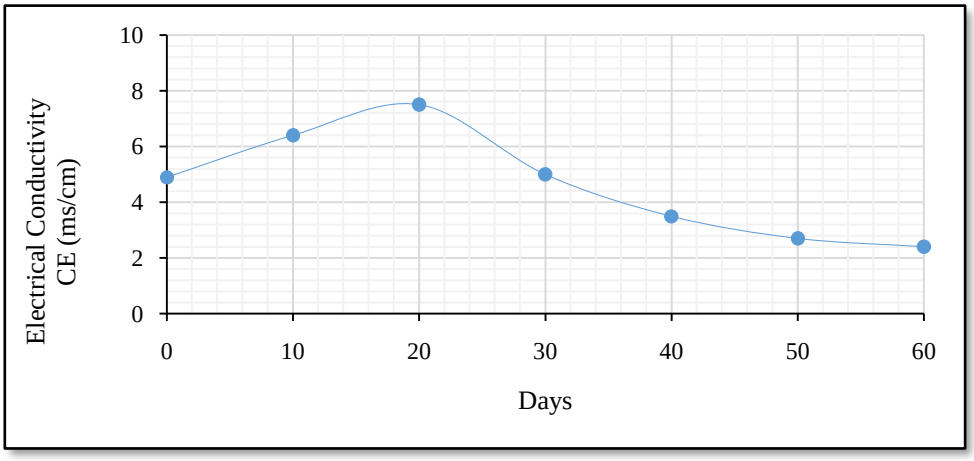

Figure 8: Evolution of the EC during the composting process

During the composting process, the microbial flora varies considerably (Figure 9). Moreover, the bacterial density is always higher than the fungal density whatever the age of the compost.

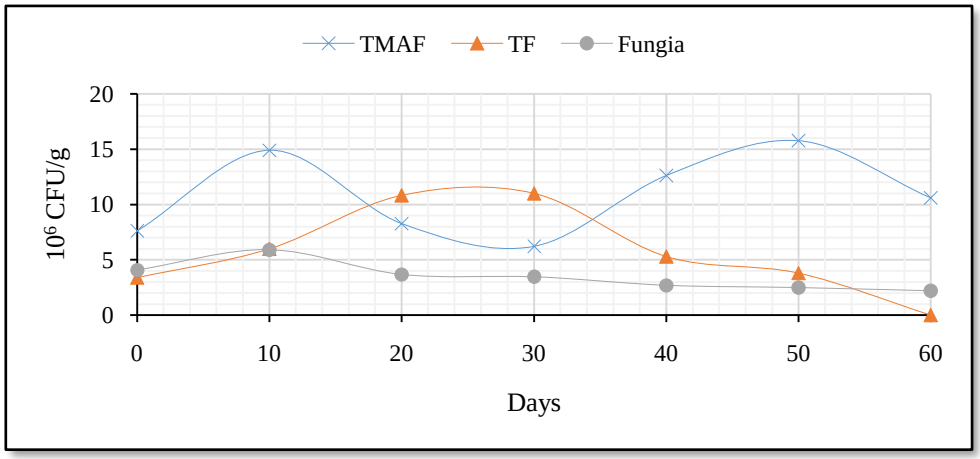

Figure 9: Evolution of microbial flora during the composting process

The beginning of the mesophilic phase is characterized by dominance of the mesophilic flora which is around $3x10^6$ CFU / g and by the fungi 370 CFU / g. According to Sifi [34], it is the fungi that first colonize the substrates and preferentially degrade fresh organic matter, but the increase in temperature leads to their decrease and their number increases from 370 to 40 CFU / g from the 20th day until the 25th day this is in agreement with the work of Greenberg et al. [35] and Ndegwa [36] who have deduced that the decline in the number of fungi is due to the installation of conditions unfavorable to their proliferation. By the 20th day, the intense activity of the micro-organisms generates a temperature rise which reaches 64.3°C., which allows the installation of the thermophilic microorganisms and there is a clear decrease in the number of mesophiles. The latter are partially inactivated or killed and, on the other hand, the number of thermophiles increases substantially and reaches $5x10^4$ CFU / g of worms on the 25th day. The thermophilic microflora thus becomes responsible for the process of degradation of the substrates during the thermophilic phase. In this context, Beffa et al. [21] find that the genus *Thermus* is among the group of thermophilic bacteria that dominates in the degradation of organic matter.

On the 40th day, there are restricted conditions due to the degradation of the substrate and consequently the decrease in temperature. This is the cooling phase, which affects mainly the thermophilic microflora and favors the environment for the resettlement of the new mesophilic microflora. These results are in agreement with other authors Tønner-Klank et al. [37] and Chroni et al. [38] which showed an intense decrease in microflora at the end of the thermophilic phase. Several other factors, such as oxygen, moisture, pH, and temperature, can affect the succession of the microbial community during composting. Paul and Clark [39] have shown that the rapid change in physicochemical conditions during composting is a selective factor of microbial succession.

Sidhu et al. [40] have also shown that the bacterial population has declined progressively with composting maturity, when nutrients and moisture content decline. This significant reduction in the number of thermophiles, argues in favor of the good progress of the composting process since their number reaches $2x10^2$ CFU / g.
In the maturation phase, the compost is repopulated by the mesophilic flora, since it reaches 3×10^5 CFU / g. These microorganisms are the most anticipated for two main reasons: they accelerate the decomposition process and give mature compost by eliminating the pathogens transported by various compounds of waste. These microorganisms continue to degrade substrates such as cellulose, lignin, etc., to obtain stable compost at the end of the cycle [9, 41, 42].

4. Phytotoxicity tests
4.1. Germination Test
The germination test is a means of assessing the toxicity associated with the incorporation of immature composts into the soil to realize the maturity of compost. The results of the germination tests on the pure or mixed compost are shown in Table 2.

Table 2: Germination test of two varieties of wheat and tomato on pure compost or mixed soil.

	Sand (control)	Sand + 1/3 Compost	Sand + 2/3 Compost	100% Compost
Wheat	70	85	57	23
Tomato	58	67	0	0

These results show that incorporation of a 25% compost dose on the soil results in a germination rate of 85% for wheat and 67% for tomatoes. On the other hand, the cultures on a substrate containing 75% to 100% of the compost are rather repressed. These results are in agreement with those reported by De hann and Abad Berjon who reported that the depressive effect of compost is not only related to the characteristics of the compost, but also to the doses applied [43, 44]. The germination therefore varies with the dose of the compost brought and the type of crop [45].

4.2. Germination index
The most significant GIs were obtained with doses of 25% and 50% aqueous extract of the different compost (Table 3). According to Zucconi et al. [16], a compost is considered non-toxic when its GI exceeds 50%. A 75% compost extract yields 77% germination index with lettuce seeds. The pure compost extract (100% EC) gave germination index percentages between 46% for lettuce. These results show that the germination index rate varies with the doses of compost extracts and with the type of the culture.

Table 3: Rate of germination indices (%) of lettuce seeds on extracts of pure compost or mixed with distilled water

Dose	100% DW	75%DW +25%CE	50%DW +50%CE	25%DW +75%CE	100%CE
Lettuce	100	77	67	61	46

DW: Distilled water ; **CE**: Compost Extract

Conclusion
In the aerobic composting process, microorganisms require oxygen to decompose the organic matter. In this study, oxygen consumption was provided by semi-continuous air injection into the bioreactor. The results show that household waste can be composted successfully within 60 days. Operational indices such as temperature, pH and released gases were very useful for assessing composting performance and reveal vigorous microbial activity. The compost produced in this study was satisfactory for its agricultural application in terms of C/N ratio and electrical conductivity as an index of its salt content. The bacterial community undergoes a change related to the availability of the nutrient substrate and the physico-chemical variations of the environment. The results obtained indicate that the development and succession of the endogenous microflora is strictly related to substrates and composting stages. In general, the composting process proposed in this study is effective because it accelerates compost degradation and maturation and requires less space for its composting.

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